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Short communication

Altered miRNA-4321 expression in maternal and foetal placenta of intrauterine growth restricted bovine foetuses.

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Abstract

Intrauterine growth restriction (IUGR) is a serious pregnancy complication caused by placental insufficiency. We previously reported that truncation of *MIMT1* gene exons 3 and 4 (*MIMT1*^{Del}) causes IUGR in cattle. Here we investigated miRNA expression in the foetal and maternal placenta tissues of *MIMT1*$^{Del/WT}$ foetuses. Small RNA next generation sequencing and quantitative PCR revealed placental tissue-specific expression of the miR-4321, known to regulate many genes involved in early foetal development. This study also indicated that maternal and foetal components of the placenta respond differently to a deleterious conceptus mutation.

Key words: bovine foetal growth restriction, miRNA expression, placenta, mammals

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1. Introduction

Intrauterine growth restriction (IUGR) is a major problem in both human [1] and animal [2] pregnancies responsible for perinatal mortality and morbidity [3] and ill health in later life [4].

The placenta is a heterocellular structure composed of foetal and maternal cells that creates an interface between mother and foetus. Balanced communication between placental cells is crucial for proper foetal development [5]. We previously reported that truncation of exons 3 and 4 of the *MIMT1* gene (MER1 repeat containing imprinted transcript 1) causes IUGR and late abortion in Ayrshire cattle [6]. We also showed that maternal placental tissues affect the penetrance of the paternally inherited mutation [7].

miRNAs are important in embryo development in human, mice and cattle [8-10], and altered expression has been implicated in several pregnancy complications [11], including intrauterine growth restriction [12]. Blocking the production of maternally inherited miRNAs by inactivation of Dicer in maturing oocytes resulted in IUGR [13], suggesting that foetal miRNAs play an important role in placental development and function, possibly via communication between the foetal and maternal placenta.

The aim of this study was to examine miRNA expression in the foetal and maternal placenta tissues of intrauterine growth restricted bovine foetuses.

2. Methods

2.1 Ethics statement

All experimental procedures involving animals were conducted in accordance with ethical standards approved by the Animal Ethics Committee of the State Provincial Office of Southern Finland (ESAVI-2010-08583/YM-230). Insemination and tissue sampling were carried out by standard veterinary protocols according to European Union Normative for Care and Use of Experimental Animals.

2.2 Tissue samples

Maternal and foetal placenta samples were collected from cows gestating *MIMT1*$^{Del/WT}$ foetuses and cows gestating wild-type foetuses terminated on the 94th ± 12 day of pregnancy, as described earlier [7]. All foetuses were gestated in wild-type cows and all were fathered by the same *MIMT1*$^{Del/WT}$ sire. Placenta samples were stored at -80°C until analysed. Two to three random placentome samples from 12 (5 males) *MIMT1*$^{Del/WT}$ (referred as IUGR) and 12 (3 males) *MIMT1*$^{WT/WT}$ (wild-type) conceptus were used for small RNA next

generation sequencing and quantitative real-time reverse transcriptase polymerase chain reaction (qPCR).

2.3 RNA extraction

20 mg of tissue samples was used for total RNA extraction using Zymo Direct-zol RNA MiniPrep kit (Zymo Research) according to the manufacturer´s protocol. The quality and quantity of isolated total RNA was measured using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific) and the fraction of small RNAs was examined on a 2100 Bioanalyzer (Agilent). The RNA integrity values (RIN) ranged from 7.9 to 9.1.

2.4 Small RNA next generation sequencing (NGS)

Small RNA NGS was performed using a TruSeq Small RNA Library Prep Kit (Illumina), as previously described [14]. The Galaxy server was used for bioinformatic analyses. KEGG pathway (Kyoto Encyclopedia of Genes and Genomes) and mRNA : miRNA interaction analyses were performed using DIANA-miRPath v3.0 and TarBase v.7.0 software [14].

2.5 Quantitative real-time PCR (qPCR)

qPCR was performed as described earlier [14]. Relative miRNA expression differences were normalised to endogenous miR-99b control. Data from IUGR and wild-type samples were compared by Students t test.

3. Results and discussion

miRNA profiling of foetal and maternal placental tissues using small RNA NGS revealed 48 foetal and 22 maternal miRNAs that were differentially expressed (FDR < 0.1) in IUGR versus normal pregnancies, Figure 1. The male and female conceptus differ in placenta structure [15]. Our analysis did not reveal a significant conceptus sex effect, possibly due to the low number of samples analysed. To identify possible roles in early development, we performed functional enrichment analysis based on their target mRNAs. The pathways most significantly enriched ($P < 0.0001$) were related to ‘oocyte meiosis’, ‘Wnt signalling pathway’ and ‘Dorso-ventral axis formation’.

Seven differentially expressed miRNAs were validated by qRT-PCR: miR-29a, miR-92b, miR-125b, miR-7641, miR-4321, miR-let-7g and miR-2895. These were selected on the basis of ranking in the list of differentially expressed miRNAs and predicted functional relevance for foetal growth and development. qPCR data confirmed differential expression of miR-4321 and miR-2895, with miR-4321 showing the greatest difference at 3.8-fold ($P < 0.01$) lower expression in IUGR than wild-type maternal placenta, Figure 2. Interestingly,

miR-432 displayed the opposite pattern of expression level in foetal placenta of IUGR, Figure 2. The other five miRNAs were not differentially expressed. Reasons for this discrepancy could include the heterocellular nature of placenta and differences in the sensitivity of the techniques.

miR-4321 has been reported to regulate several target genes involved in foetal growth and development. These include *TRO* (trophinin), which mediates adhesion of trophoblastic cells and epithelial cells of the endometrium and participates in cell adhesion during embryo implantation [16]. Others include *STOX1 and DYRK1A*, mutations of which are associated with pre-eclampsia with foetal growth retardation [17]. Possibly the most important miR-4321 target is *PTGER3* (prostaglandin E receptor 3), which is mainly expressed in extravillous trophoblast of human placenta and involved in trophoblast invasion and placentation. Increased *PTGER3* expression has been observed in recurrent pregnancy loss in humans [18] and in IUGR rats [19]. It is noteworthy that none of these genes were identified as differentially expressed in our earlier study [7].

Thus, accurate measurement of placental miR-4321 expression may be a biomarker for detection of pregnancies in risk. However, this would require robust and reliable means of distinguishing maternal and foetal placental cells, as each may respond differently to foetal genotype.

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Figure legends

Figure 1. Heatmaps showing the top differentially expressed miRNAs in foetal (a) and maternal (b) placental tissues of wild-type and IUGR bovine foetuses. The expression data are presented based on the normalised Log2-transformed fold change values and the *P*-value. m – male, f – female conceptus.

Figure 2. Relative miRNA expression in a) placenta fetalis and b) placenta materna of wild-type (n= 12) and IUGR (n= 12) bovine foetuses. Values are means and standard deviations normalised to miR-99b. * $P < 0.05$, ** $P < 0.01$.

Supporting information

Table S1. List of qPCR primers.

Table S2. Differentially expressed miRNAs in maternal and foetal placenta tissues of IUGR bovine foetuses.

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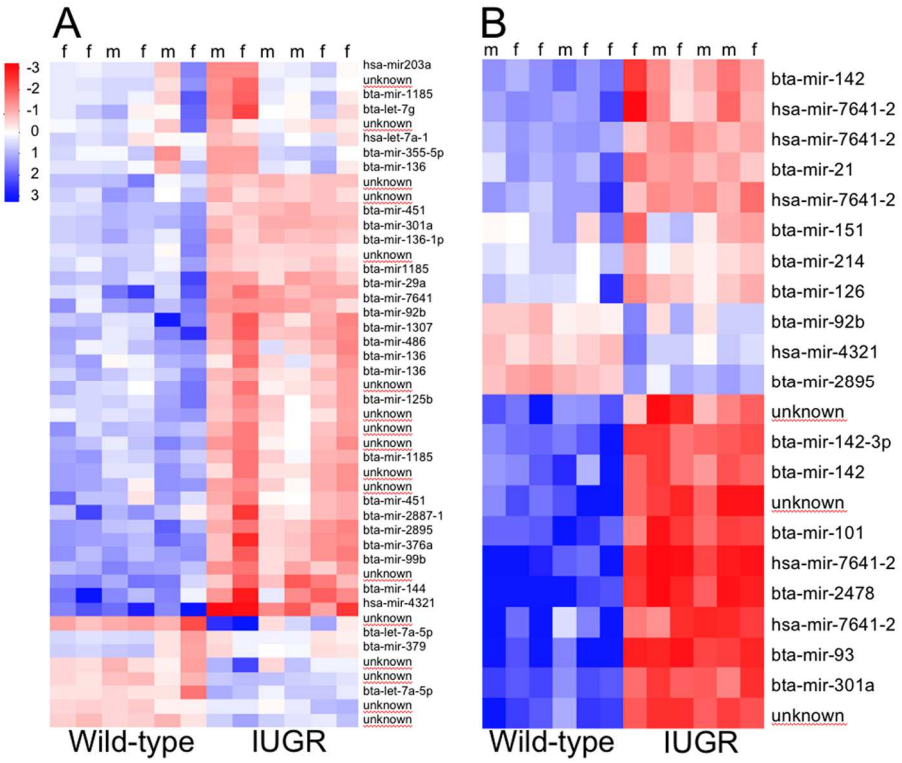

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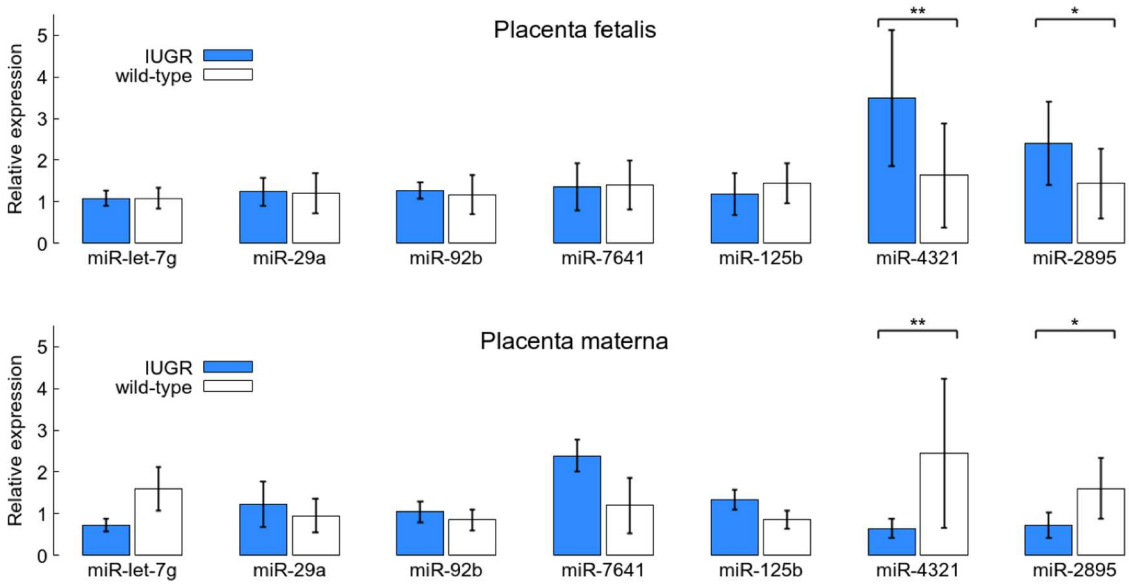

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Highlights

- Aberrant expression of miR-4321 in bovine IUGR placenta.
- MiR-4321 regulate genes involved in early foetal development.
- Placental fetalis and materna respond differently to conceptus genotype.

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